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PTO/SB/05 (4/98)
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	SMAR.P001
First Inventor or Application Identifier	MARDH
Title	Screening Method for Gastritis
Express Mail Label No.	EL556132085US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

1. [✓] * Fee Transmittal Form *(e.g., PTO/SB/17)* *(Submit an original and a duplicate for fee processing)*
2. [✓] Specification [Total Pages 31] *(preferred arrangement set forth below)*
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [✓] Drawing(s) *(35 U.S.C. 113)* [Total Sheets 4]
4. Oath or Declaration [Total Pages]
   - a. [✓] Newly executed (original or copy)
   - b. [ ] Copy from a prior application (37 C.F.R. § 1.63(d)) *(for continuation/divisional with Box 16 completed)*
     - i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

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5. [ ] Microfiche Computer Program *(Appendix)*
6. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   - a. [ ] Computer Readable Copy
   - b. [ ] Paper Copy (identical to computer copy)
   - c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. [ ] Assignment Papers (cover sheet & document(s))
8. [ ] 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
9. [ ] English Translation Document *(if applicable)*
10. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
11. [ ] Preliminary Amendment
12. [✓] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
13. [✓] * Small Entity Statement(s) *(PTO/SB/09-12)* [ ] Statement filed in prior application, Status still proper and desired
14. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
15. [ ] Other: .....

16. **If a CONTINUING APPLICATION**, *check appropriate box, and supply the requisite information below and in a preliminary amendment:*
    [ ] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application No: _____/_____
    Prior application information: Examiner _____ *Group / Art Unit:* _____
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Name (Print/Type)	NANCY J. PARSONS	Registration No. (Attorney/Agent)	40,364
Signature	Nancy J. Parsons	Date	10/03/00

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Patent fees are subject to annual revision on October 1.
These are the fees effective October 1, 1997.
Small Entity payments must be supported by a small entity statement, otherwise large entity fees must be paid. See Forms PTO/SB/09-12. See 37 C.F.R. §§ 1.27 and 1.28.

TOTAL AMOUNT OF PAYMENT ($) 435

Complete if Known	
Application Number	TBA
Filing Date	10-03-2000
First Named Inventor	MARDH
Examiner Name	
Group / Art Unit	
Attorney Docket No.	SMAR.P-001

METHOD OF PAYMENT (check one)

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☐ Charge Any Additional Fee Required Under 37 C.F.R. §§ 1.16 and 1.17

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2. ☑ **Payment Enclosed:**
☑ Check ☐ Money Order ☐ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	790	201	395	Utility filing fee	355
106	330	206	165	Design filing fee	
107	540	207	270	Plant filing fee	
108	790	208	395	Reissue filing fee	
114	150	214	75	Provisional filing fee	

SUBTOTAL (1) ($) 355

2. EXTRA CLAIM FEES

		Extra Claims		Fee from below	=	Fee Paid
Total Claims	13	-20** =	0	X	=	
Independent Claims	5	- 3** =	2	X 40	=	80
Multiple Dependent					=	

***or number previously paid, if greater; For Reissues, see below*

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	22	203	11	Claims in excess of 20
102	82	202	41	Independent claims in excess of 3
104	270	204	135	Multiple dependent claim, if not paid
109	82	209	41	** Reissue independent claims over original patent
110	22	210	11	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 80

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet.	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	950	217	475	Extension for reply within third month	
118	1,510	218	755	Extension for reply within fourth month	
128	2,060	228	1,030	Extension for reply within fifth month	
119	310	219	155	Notice of Appeal	
120	310	220	155	Filing a brief in support of an appeal	
121	270	221	135	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,320	241	660	Petition to revive - unintentional	
142	1,320	242	660	Utility issue fee (or reissue)	
143	450	243	225	Design issue fee	
144	670	244	335	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	790	246	395	Filing a submission after final rejection (37 CFR 1.129(a))	
149	790	249	395	For each additional invention to be examined (37 CFR 1.129(b))	

Other fee (specify) _____

Other fee (specify) _____

* Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)

SUBMITTED BY				Complete (if applicable)	
Typed or Printed Name	NANCY J. PARSONS			Reg. Number	40,364
Signature	Nancy J. Parsons	Date	10/03/00	Deposit Account User ID	

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STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(b))--INDEPENDENT INVENTOR	Docket Number (Optional) SMAR.P001

Applicant, Patentee, or Identifier: SVEN MARDH AND ERIK MARDH

Application or Patent No.:

Filed or Issued: OCTOBER 3, 2000

Title: Screening Method for Gastritis

As a below named inventor, I hereby state that I qualify as an independent inventor as defined in 37 CFR 1.9(c) for purposes of paying reduced fees to the Patent and Trademark Office described in:

- [XX] the specification filed herewith with title as listed above.
- [ ] the application identified above.
- [ ] the patent identified above.

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Each person, concern, or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

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Separate statements are required from each named person, concern, or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

Sven Mårdh	Erik Mårdh	
NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
Sven Mårdh	Erik Mårdh	
Signature of inventor	Signature of inventor	Signature of inventor
2000-08-14	2000-08-14	
Date	Date	Date

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Screening Method for Gastritis

BACKGROUND OF THE INVENTION

Dyspepsia, or indigestion, is a common diagnosis in primary health care, but with poorly defined management. The annual prevalence of dyspepsia in the United Kingdom (UK) is about 25%, and in primary health care it accounts for 3-4% of the consultations (Harris, A., Eur J Gastroenterol Hepat 1999; 11 (Suppl 1): S31-5). Among the chronic disorders of the upper gastrointestinal tract are those which fall under the general category of gastritis. Gastritis is an inflammation of the stomach mucosa which is manifested by a broad range of poorly-defined symptoms such as indigestion, "heart burn" and excessive eructation. The typical means used to diagnose gastrointestinal disorders depends on such factors as the nature and severity of symptoms, the overall health of the individual, the medical history of the patient, the need for a specific diagnosis in order to implement a treatment with reasonable likelihood of success, and the availability of diagnostic devices.

Esophagogastroduodenoscopy (EGD) with histopathological examination of biopsies is the gold standard to determine the status of the gastric and duodenal mucosa. This examination is safe, accurate and sometimes indispensable, e.g., in the older age group and especially in the presence of alarm symptoms such as weight loss, anorexia, dysphagia, or gastrointestinal blood loss. The demand for upper endoscopy is increasing and in the UK approximately 0.5% of the population undergo this examination each year (Working Party of the Clinical Services Committee of the British Society of Gastroenterology, Provision of gastrointestinal endoscopy and related services for a district general hospital. Gut 1991; 32: 95-105; Gear, M.W.L., and Wilkinson, S.P., Br J Hosp Med 1989; 41: 438-44). Without EGD and visual inspection of the mucosa, gastritis is difficult to diagnose. However, EGD is expensive,

inconvenient for the patient, and generally not recommended for children or patients with severe cardiopulmonary disease. Thus, for patients not having severe symptoms, a precise diagnosis of a gastrointestinal disorder might not be attempted. Such patients may simply be treated with conventional therapies, such as with antacids or drugs which inhibit stomach acid secretion. While such therapies might provide temporary relief of the symptoms, a cure is not often achieved. More effective treatments generally depend on a better diagnosis of the actual underlying gastrointestinal disorder. For example, many gastrointestinal disorders are mediated by bacterial infection of the mucosa, in which case treatment of the bacterial infection would most likely be required to effectively treat the manifested gastrointestinal disorder.

There is a need for a simple pre-gastroscopic screening method to reduce the endoscopy workload, and attempts have been made in this direction with some success in patients with uncomplicated simple dyspepsia (Bodger, K., et al., Scand J Gastroenterol 1999; 34: 856-63; and Moayyedi, P., et al., Eur J Gastroenterol Hepatol 1999; 11:1245-50). In young dyspeptic patients (< 40 years) screening for Helicobacter pylori (H. pylori) infection and a treatment strategy based on the presence of an infection reduces the endoscopy workload. This strategy appears as effective as an endoscopy-based strategy in reducing dyspeptic symptoms, dyspepsia consultation rates and the prescription of anti-secretory drugs (Moayyedi, P., et al., Eur J Gastroenterol Hepatol 1999; 11:1245-50). In the elderly with dyspepsia, however, the prevalence of gastritis and its consequences are considerably higher pointing at EGD as the initial diagnostic step.

The inflamed gastric mucosa transmits specific information to the blood stream that allows diagnosis of gastritis by serologic analysis. The morphology and cellular composition of the mucosa vary between the acid secreting corpus and the antrum. This may aid to distinguish corpus and pangastritis from antral gastritis. A number of serological markers have been described. Infection with H. pylori is the major cause of chronic gastritis, duodenal ulcer, mucosa associated lymphoid tissue (MALT)

lymphoma and gastric cancer (Chiba, N., et al., Can Fam Physician 1998; 44: 1481-8; Genta, R.M., Gut 1998; 43: 35-8; Coyle, W.J., et al., Gastrointest Endosc 1998; 48: 327-8; Lee, B.M., et al., Jpn J Cancer Res 1998; 89: 597-603), and antibodies to various H. pylori antigens can easily be detected in the blood (Bodger, K., et al., Scand J Gastroenterol 1999; 34: 856-63; Moayyedi, P., et al., Eur J Gastroenterol Hepatol 1999; 11:1245-50). This infection is sometimes associated with an autoimmune reaction leading to atrophy of the corpus mucosa (Ozasa, K., et al., Dig Dis Sci 1999; 44: 253-6). A common feature of gastric autoimmunity and frequently several other autoimmune diseases, e.g., thyroiditis, insulin dependent diabetes mellitus and sometimes rheumatoid arthritis, is the occurrence of parietal cell autoantibodies (Bech, K., et al., Acta Endocrinol 1991, 124: 534-9; Barrio, R., et al., Pediatr Endocrinol Metab 1997, 10: 511-6; Datta, A., et al., Indian J Med Res 1990, 92: 228-32; Mårdh, S., et al., Scand J Gastroenterol 1991, 26: 1089-96). The parietal cell H,K-ATPase α- and ß-subunits were found to be the major autoantigens in autoimmune atrophic gastritis (Karlsson, A., et al., J Clin Invest 1988, 81: 475-9; Song, Y.H., et al., Scand J Gastroenterol 1994, 29:122-7; Ma, J.Y., et al., Scand J Gastroenterol 1994, 20: 790-4). A low titre of H,K-ATPase antibodies is normally found in healthy individuals due to the normal turn-over of parietal cells. In patients with inflamed corpus mucosa the titre may be increased.

Pepsinogen I (PGI) is secreted by the chief and mucous neck cells of the corpus mucosa into the lumen of the stomach but a small fraction (about 1%) leaks into the blood stream (Baron, J.H., Clinical tests of gastric secretion: History, Methodology and Interpretation. (1978) London: Macmillan). Increased serum concentrations of PGI are frequently found in patients with duodenal ulcer (Samloff, I.M., et al., Gastroenterol 1975 Jul, 69(1): 83-90). In patients with pernicious anaemia due to severe atrophy of the corpus mucosa serum PGI is significantly reduced (Samloff, I.M., et al., Gastroenterol 1982 Jul, 83(1 Pt 2): 204-9).

Existing, non-invasive methods of detecting gastrointestinal disorders include monitoring blood flow to the affected region to detect inflammation (US 5,524,622). A

significant disadvantage of this method is the requirement of injecting multiple substances into the patient followed by the detection by gamma camera. Additionally, the method only detects inflammation, and does not address the underlying cause of any inflammation. Other methods of detecting gastrointestinal disorders include assays for individual analytes such as pepsinogen (US 5,879,897) or Helicobacter pylori (US 5,814,455; 6,067,989;6,068,985; 6,090,611). Other serological markers are gastrin (Borch, K., et al., Scand J Gastroenterol 1997, 32:198-202), pepsinogen II (Carmel, R., Am J Pathol 1998, 90: 442-5), intrinsic factor antibodies (Waters, H.M., et al., J Clin Pathol 1989, 42: 307-12) and pepsinogen antibodies (Mårdh, S., et al., Acta Physiol Scand 1989, 136: 581-7). Although each one of these markers may be used to diagnose changes in the gastric mucosa, the overlap between healthy subjects and patients is great, and even greater among the various subgroups of patients. Therefore none of all these markers alone is sufficient for a reliable diagnosis.

SUMMARY OF THE INVENTION

The instant invention provides a screening method for gastritis in its various forms involving the evaluation of assay results for H,K-ATPase antibodies, H. pylori antibodies, and serum pepsinogen I concentration. The analysis of multiple analytes associated with gastritis provides a reliable indication of various subgroups of gastritis.

In one embodiment, the invention comprises a method for evaluating a blood sample from a patient to assess the likelihood that the patient has gastritis. In this method, the blood sample is tested for the presence of antibodies specific for H,K-ATPase, antibodies specific for Helicobacter pylori, and for the concentration of pepsinogen I. The levels of these analytes are compared with levels from individuals without gastritis, and the presence of H,K-ATPase antibodies, Helicobacter pylori antibodies or altered pepsinogen concentration is indicative of the patient having gastritis.

In another embodiment, the screening method for gastritis comprises determining H,K-ATPase antibodies, Helicobacter pylori antibodies, and the level of pepsinogen I in a biological sample from a patient suspected of suffering from gastritis, and comparing the level of the analytes to levels in normal individuals of the same species. Altered levels in the sample compared to the level in normal individuals of the same species is indicative of gastritis.

In a further embodiment, the screening method for gastritis comprises determining the levels of at least two indicators in a biological sample from a mammalian patient, the indicators selected from the group consisting of H,K-ATPase antibodies, Helicobacter pylori antibodies, and the level of pepsinogen I. The levels of indicators are compared to levels in normal mammals of the same species, and levels of at least two indicators in the sample which differ significantly from the level in normal mammals of the same species is indicative of gastritis. Determining the levels of the indicators may be achieved by immunoassays.

In a further embodiment of the invention, the screening method includes an additional indicator comprising the level of pepsinogen I multiplied by the level of Helicobacter pylori antibodies, which is also compared to the same indicator in normal mammals of the same species.

In another embodiment, the invention comprises a method of determining which one out of several subgroups of gastritis a patient has. H,K-ATPase antibodies, Helicobacter pylori antibodies, and the level of pepsinogen I, and the product of the level of pepsinogen I multiplied by the level of Helicobacter pylori antibodies are measured, and the results are compared to a flow-chart which shows various levels of the different analytes and which subgroup of gastritis is associated with them.

In an additional embodiment of the invention, a kit is provided for performing the screening method. The kit preferably comprises reagents suitable for detecting H,K-ATPase antibodies, Helicobacter pylori antibodies, and the level of pepsinogen I. The reagents for each of the three assays are preferably present in amounts to perform equal numbers of the three assays. Preferably, the method of detection is

immunoassay, and the reagents include pepsinogen I antibodies, H,K-ATPase and Helicobacter pylori proteins, or peptides thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1D are graphs showing the levels of H,K-ATPase antibodies, H. pylori antibodies, pepsinogen I, and result of H. pylori antibody level multiplied by pepsinogen I concentration, respectively, for individuals with normal gastric mucosa (N) and those with duodenal ulcer (DU), atrophic gastritis (AG) and gastritis with pernicious anemia (PA).

FIG. 2 is a flow chart depicting a testing evaluation scheme.

FIGS. 3A-3D are pie charts showing the four major groups identified by the screening tests of the instant invention for the test groups N, DU, AG, and PA.

FIGS. 4A-4D are pie charts showing the four major groups identified by the screening tests of the instant invention for the general population.

DETAILED DESCRIPTION OF THE INVENTION

Gastritis and dyspepsia are common entities in primary health care but with poorly defined management. The aim of the present invention is to provide a serologic screening test for gastritis. Esophagogastroduodenoscopy (EGD) with biopsy and histological examination requires a skillful and experienced staff and it is presently the only reliable technique for diagnosing gastritis, benign ulcer and neoplasia. The last two groups are generally closely associated with chronic gastritis. Previous investigations often concluded that serology has a limited diagnostic value. However, the instant evaluation scheme for analyzing serology data is useful as a pregastroscopic screening of dyspepsia, irrespective of the age of the patient, which is a remarkable improvement

compared with previous reports (Bodger, K., et al., Scand J Gastroenterol 1999, 34: 856-63; Moayyedi, P., et al., Eur J Gastroenterol Hepatol 1999, 11:1245-50). The immune system and chemical signalling from the inflamed gastric mucosa provide serum analytes and diagnostic possibilities for detection of gastritis.

Assay methods for determining the level of H,K-ATPase antibodies, Helicobacter pylori antibodies and pepsinogen I concentration are known. In a preferred embodiment of the instant invention, the method of determining the level of the analytes is by immunoassay. The immunoassay may be any of the well-known methods, including, but not limited to, enzyme-linked-immunosorbent assays (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), immunoprecipitation (IP), and optical or electrochemical detection of immuno-ligand interaction. In a preferred embodiment, the immunoassay is one in which the antigen is immobilized on a solid support, sample is added, followed by labelled antibody.

One embodiment of the invention is a kit for screening for gastritis. The kit comprises the reagents required to perform assays for at least two indicators selected from the group consisting of H,K-ATPase antibodies, Helicobacter pylori antibodies, and the level of pepsinogen I. In a preferred embodiment, the assays are immunoassays, and the kit comprises analytes immobilized on one or more solid supports, such as a microtitre plate, strip of paper, nitrocellulose or other suitable material. Labeled antibody is included for detection. The kit preferably contains reagents for each assay in amounts sufficient to perform equal numbers of the different assays. For example, a kit may contain sufficient reagents to perform ten H,K-ATPase assays, ten Helicobacter pylori assays and ten pepsinogen assays. The kit may additionally comprise analyte-specific antibodies, labelling reagents, positive and negative controls and wash solutions.

The immunoassay methods are based on analyses of a blood sample (or plasma or serum) from patients; the autoantibodies against H,K-ATPase, antibodies against Helicobacter pylori and concentration of pepsinogen are assayed. All of these

analytes serve as markers of an inflammatory condition in the gastric mucosa. The methods of detecting the analytes, such as immunoassays, are well-known in the art.

The assay results are analysed by a new grouping procedure in which the results are compared with reference values from healthy individuals from the normal population. A mathematical expression (the product of pepsinogen concentration multiplied by the titre of Helicobacter pylori antibodies) is essential to identify one particular group of patients. Other mathematical procedures may be used provided they achieve determination of a useful grouping of gastritis patients. This grouping procedure diagnoses inflammatory conditions in the gastric mucosa that previously have required the more costly and complicated gastroscopy with histopathological examination of biopsies from the mucosa.

The reference values from healthy individuals from the normal population may be standardized for each assay. In this embodiment, the test results are compared to standardized reference values in order to determine the patient's likelihood of having gastritis. A kit would contain a list of the standardized reference values for each assay. In an alternative embodiment, the reference values are obtained by testing a normal control along with the patient samples. In this embodiment, a kit would contain a normal control and standards. When performing the assays, one would run the assay on the control and standards at the same time the patient samples are tested. The assay results for the control and standards would then be compared to the results for the patient samples.

Sera from subjects examined with gastroscopy and biopsy were analysed for H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I. The diagnoses were normal gastric mucosa (n=50), duodenal ulcer (n=53), and atrophic corpus gastritis with (n=50) or without pernicious anaemia (n=46). An evaluation scheme (flow chart) was constructed to optimise the diagnostic agreement between serology and gastric mucosal morphology. Four major serologic groups and thirteen subgroups were obtained with an over-all sensitivity to detect gastritis of 98% (146/149) (95% CI 94-100%) and a specificity of 84% (42/50) (95% CI 71-93%). Additional sera

from 483 subjects from the general population were grouped by serology. The overall sensitivity to detect gastritis in this population was 88% (211/240) (95% CI 83-92%) with a specificity of 81% (196/243) (95% CI 75-85%). There was a good agreement between serology and the gastric mucosal morphology both in the groups used for developing the evaluation scheme and in the sample of the general population. Thus, serology is appropriate for initial identification of subjects with a normal gastric mucosa, those who qualify for eradication of Helicobacter pylori, and those who are at risk of developing malignancy and therefore require gastroscopic examination.

In reading the assay results, altered levels of the analytes in a patient sample as compared to normal control values is indicative of the patient having gastritis. By "altered" is meant levels either significantly above or significantly below the levels of the normal control. What is significant depends on the accuracy and precision of the specific test performed and may be determined empirically without undue experimentation. Additionally, the levels of multiple analytes in a patient sample are compared to normal control values in order to obtain a more accurate determination of whether or not the patient has gastritis. For example, in general, patient levels of H,K-ATPase antibodies and Helicobacter pylori antibodies above normal control levels is indicative of gastritis, while patient levels of the pepsinogen concentration either significantly above (duodenal ulcer) or below (severe atrophy of the corpus mucosa) normal control levels may be indicative of gastritis. The pepsinogen concentration multiplied by the Helicobacter pylori antibody titre either significantly above or significantly below the normal control may be indicative of gastritis. In order to determine the likelihood of the patient having gastritis and/or to determine the subgroup of the patient's gastritis, the combination of multiple assay results are compared to normal control values. Because some analytes may be either higher or lower than normal control levels and still be indicative of gastritis, the comparison of levels of multiple analytes in a patient sample to normal control levels of the same analytes provides a more accurate determination of gastritis.

With histomorphological examination as the gold standard, the present invention demonstrates that a combination of serologic assays detects 87% (196/225, group 1A in FIG. 2; Table 2) of subjects with normal gastric mucosa in a sample of the general population and in the study groups it was 84% (42/50, group 1A; Table 1). In the study groups, serology detected 91% (48/53, group 2) of subjects with duodenal ulcer and 84% (81/96, groups 3 plus 4) of subjects with corpus predominant atrophic gastritis with or without pernicious anaemia. All subjects with pernicious anaemia were obtained in group 4. In the population sample, groups 2A-D comprised 15%. Among these, only 3% had a normal mucosa, while antral gastritis with atrophy and pangastritis overall comprised 88%. All subjects in groups 2A-D were H. pylori-positive. It is therefore suggested that subjects aged 40-50 years or less (Bodger, K., et al., Scand J Gastroenterol 1999, 34: 856-63; Moayyedi, P., et al., Eur J Gastroenterol Hepatol 1999, 11:1245-50) that are obtained in groups 2A-D may be treated according to the current recommendations for peptic ulcer disease.

Only 12 subjects in the population sample were obtained in group 4A-4C; one had non-atrophic corpus predominant gastritis and the remaining 11 atrophic corpus predominant gastritis. Thus, subjects belonging to group 4 should be recommended EGD due to the increased risk of malignancy.

In the population sample there were 12% (29/225) with normal gastric mucosa, but abnormal results of the serological analysis. There are probably several explanations for this discrepancy; some results of serum analytes may reflect a previous inflammatory condition, or the ELISAs may be more sensitive than the most experienced examiner to detect small changes in the mucosa.

In conclusion, EGD with biopsy remains the gold standard for an accurate diagnosis of the status of the gastric mucosa. However, as the following examples show, serology is a good complement when combined with the patient's symptomatology and medical history. It functions as a "serologic biopsy". Therefore, serologic assays are well-suited for pregastroscopic screening of dyspepsia to identify: (a) patients with normal gastric mucosa (group 1A), (b) H. pylori-positive patients with

high levels of serum PGI and high "H.p. x PGI -factor" (group 2), and (c) patients with corpus predominant atrophic gastritis (groups 3 plus 4). Patients in (a) may be examined further for "non-acid-related" disorders, those in (b) younger than 40-50 years may be treated according to the recommendations for peptic ulcer disease, and in cases with unsuccessful treatment, the patients should be referred to EGD. Patients in (c) are at a higher risk of developing gastric malignancy and should therefore be referred to EGD.

The described serologic assays and the evaluation procedure are simple and may be performed in any clinical laboratory with some experience in immunoassays. They provide a reduction in the endoscopic workload, are beneficial for the patient, provide a valuable diagnostic tool for the doctor and are cost-efficient.

EXAMPLE 1

For the initial evaluation of the serologic results, sera from four groups of subjects examined endoscopically and histologically were selected; 50 subjects with normal gastric mucosa (N) (38 males and 12 females, median age 63 years, range 37-80), 53 subjects with acute duodenal ulcer (DU) (39 males and 14 females, median age 52 years, range 20-79), 46 subjects diagnosed as having mild to severe corpus predominant atrophic gastritis (AG) (23 males and 23 females, median age 68 years, range 40-82), and 50 subjects with corpus predominant atrophic gastritis with pernicious anaemia (PA) (23 males and 27 females, median age 68 years, range 40-83). The criteria for the diagnosis of pernicious anaemia which included a Schilling test showing intrinsic factor deficiency have previously been given (Borch, K., et al., Scand J Gastroenterol 1984, 19: 154-60).

EXAMPLE 2

A sample of 483 subjects (266 males and 217 females, median age 65, range 37 to 85 years) randomly selected from a general population in Sweden was examined with EGD with biopsy and blood sampling. Results of this study have recently been

published (Borch, K., et al., Dig Dis Sci, 2000, 45: 1322-29). In biopsy specimens, gastritis was classified according to the Sydney system into antrum predominant-, corpus predominant- and pangastritis with or without atrophy and with or without presence of H. pylori (Price, A., J Gastroenterol Hepatol 1991, 6: 209-22; Dixon, M.F., et al., Am J Surg Pathol 1996, 20: 1161-81). EGD was performed as previously described (Borch, K., et al., Dig Dis Sci, 2000, 45: 1322-29). Three biopsies were taken from the gastric body (major-, anterior-, and posterior aspect), and the antrum within 3 cm of the pylorus.

EXAMPLE 3

Preparation of antigens

H,K-ATPase was prepared from pig gastric mucosa as previously described (Mårdh, S., et al., Scand J Gastroenterol 1991, 26: 1089-96). The binding of autoantibodies against the H,K-ATPase in this porcine antigen preparation was similar to that of the human antigen (Song, Y.H., et al., Scand J Gastroenterol 1994, 29: 122-7; Ma, J.Y., et al., Scand J Gastroenterol 1994, 20: 790-4; Karlsson, F.A., et al., Clin exp Immunol 1987, 70:604-10). The vesicular membranes enriched with H,K-ATPase were treated at a low concentration of detergent (0.13% (w/v) of n-octylglucoside, or 0.06% (w/v) of sodium dodecylsulfate) to remove loosely attached proteins, e.g., pepsin/pepsinogen, and then stored at -70°C in sucrose/Hepes-Tris buffer, pH 7.4. Antigens of H. pylori were prepared from five strains (CCUG 17874, 25, 66, 1139 and 253) as described by Ma et al. (Ma, J.Y., et al., Scand J Gastroenterol 1994, 29: 961-6).

EXAMPLE 4

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed essentially as described (Ma, J.Y., et al., Scand J Gastroenterol 1994, 29: 961-6) using Nunc-Immuno® plates (Maxisorp®, Nunc, Roskilde, Denmark) coated with 50 µl of indicated antigen preparations (5 µg/ ml) in 50

mM sodium carbonate buffer, pH 9.8, and incubated overnight at 4°C. The wells were sequentially incubated with sera diluted 1:100 in phosphate-buffered saline containing 0.05% (v/v) Tween 20 (PBS-T), biotinylated goat anti-human IgG (Amersham International PLC, Amersham, UK), streptavidin (Amersham International PLC, Amersham, UK), and biotinylated alkaline phosphatase (Boehringer-Mannheim Biochemicals, Mannheim, Germany). Finally, 100 µl of p-nitrophenyl phosphate (Sigma, St Louis, MO, USA) at 1 mg/ ml in 50 mM sodium carbonate buffer, pH 9.8, was added. The absorbance was read continuously at 405 nm (kinetic ELISA) using a computerized ELISA reader (Vmax®, Molecular Devices, CA, USA). All incubations were performed with continuous shaking and the plates were washed three times with PBS-T between each incubation step. Each serum sample was analyzed in duplicates, and in each immuno plate positive and negative standards were included. The reading of the optical density (mOD per min) for each sample was related to the positive standard on each immuno plate, and the data are presented as relative titres of antibody. The coefficient of variation of the positive and negative standards were 5.7 ± 2.9 and 8.1 ± 3.5 (M+SD), respectively.

EXAMPLE 5

Analysis of serum pepsinogen I (PGI)

The assay was based on a noncompetitive sandwich technique using a horseradish peroxidase (HRP)-labeled antibody specific for PGI to detect PGI bound to a stationary antibody. The latter antibody was immobilized on a microtitre plate and had affinity for a different antigenic site in PGI than the enzyme-labeled antibody.

Assay of serum pepsinogen I (PGI) was carried out using Gastroset PGI (Gastroset PGI Cat. No. 67882, Orion Diagnostica, Espoo, Finland) according to the manufacturer's instructions. Aliquots of 20 µL of standards, control and serum sample were added in duplicate into microtitre wells precoated with stationary pepsinogen I antibody. Assay buffer (100 µL) was added and the microtitre wells incubated for 30 minutes, washed twice, and then incubated for another 30 minutes with horseradish

peroxidase-labeled PGI (HRP-PGI) antibody diluted 1:100 with assay buffer. The wells were washed four times and then incubated with substrate solution for 30 minutes; the reaction was stopped and color development measured in an ELISA-reader.

Statistical Analysis

Results are presented as median and interquartile range (25th-75th percentiles). Proportions (percentages) are given with 95 percent confidence interval (CI), when considered relevant. Wilcoxon's signed-rank test was used to evaluate differences between pairs of patient groups. The level of significance was $p<0.05$.

EXAMPLE 6

Assays of sera from the study groups N, DU, AG and PA

Enzyme-linked immunosorbent assay - Sera of four groups of subjects that had their diagnosis at endoscopy and histological examination of biopsies were analyzed for H,K-ATPase antibodies (FIG. 1A), H. pylori antibodies (FIG. 1B), and pepsinogen (PGI) (FIG. 1C). The product *f*= [relative titre of H. pylori antibodies *x* PGI] is presented in FIG. 1D. The groups included subjects with a normal gastric mucosa (N, n=50), duodenal ulcer (DU, n=53), mild to severe corpus predominant atrophic gastritis (AG, n=46), and pernicious anaemia (PA, n=50). The results are presented as box plots with median and interquartile range (25th-75th percentiles, Hspread). Values outside the inner and outer fences are plotted with asterisks and open circles, respectively, and the upper fences are defined as the interquartile range + 1.5 Hspread and + 3 Hspread, respectively (SYSTAT® manual). Values outside the y-axis are presented in parenthesis. Significance is denoted by ** ($p<0.01$) and *** ($p<0.001$); n.s. not significant.

H,K-ATPase antibodies - In the study group constituting subjects with normal gastric mucosa (N), the median H,K-ATPase antibody titre was 2.0 (range 0.7-9.0; FIG. 1A). Corresponding values in the DU, AG, and PA groups were 9.3 (range 1.6-82.7), 3.8 (range 0.8-137) and 32.3 (range 1.4-128), respectively ($p< 0.001$ vs. normals).

Helicobacter pylori antibodies - The median titre of H. pylori antibodies in group N was 1.0 (range 1.0-4.5; Fig. 1B). Corresponding values in the DU, AG, and PA groups were 47.5 (range 4.1-136), 18.0 (range 1.0-53.0) and 3.5 (range 1.3-27.2), respectively ($p<0.001$ vs. normals).

Serum pepsinogen I - The median value of serum PGI in group N was 66.3 µg per L (range 21.3-163; Fig. 1C). Corresponding values in the DU, AG, and PA groups were 149 µg per L (range 47.2-500), 31.8 µg per L (range 2.6-127), and 4.4 µg per L (range 0-61.0), respectively ($p<0.001$ vs. normals).

The factor f = (titre of H. pylori antibodies x serum pepsinogen I). Although the patient groups differed significantly using the three types of serologic parameters, there was an overlap. The titres of H. pylori antibodies and the PGI concentrations were high in the DU group and low in the PA group. Therefore, in an attempt to distinguish better between the different groups, the products (f) of the analytical results from the ELISAs of H. pylori antibodies times the PGI were evaluated (Fig. 1D). All groups were significantly different from the others ($p<0.01$ or $p<0.001$, Fig. 1D). The median value of f in group N was 77.5 (range 24.0-344). In the DU group it was 8105 (range 193-40303; $p< 0.001$ vs. normals), in AG 500 (range 5.0-3901; $p<0.001$ vs. normals), and in PA 12.0 (range 0-915; $p<0.01$ vs. normals).

EXAMPLE 7

Evaluation scheme (flow chart) for serologic diagnosis

The status of the gastric mucosa was determined by means of histopathological examination of biopsy sections. This made possible a comparison of the gold standard with the results of serologic analyses. A diagnostic evaluation scheme was developed (FIG. 2). The discriminating levels of this scheme were optimized using the results of the serologic analyses (after omitting serologic outliers) in the study groups N, DU, AG and PA. The SYSTAT® software was used to achieve a maximal resolution between the patient groups . The "analytes" were H, K-ATPase antibodies (HK), H. pylori antibodies (HP), s-pepsinogen I (PGI), and *f*= (titre of H. Pylori antibodies x serum

pepsinogen I) (HP*PGI). This scheme sorted the individual sera into the serologic groups 1-4 and their subgroups.

The evaluation scheme was applied to the analytical results from each individual serum which was sorted and grouped accordingly. Table 1 shows the serologic grouping of the four study groups: normal (N), duodenal ulcer (DU), corpus predominant gastritis without pernicious anaemia (AG) and pernicious anaemia (PA). The scheme for serologic diagnosis given in FIG. 2 was applied to the serological data.

The grouping resulted in the serologic subgroups 1A - 4C. The discriminating levels are indicated for the selection pathway in each subgroup and the median value of each group is indicated in parenthesis. The distribution of the study groups, sex and age in each serologic group are presented. In group 1A, 84% (42/50) of the subjects in group N were obtained. Some sera from subjects in group N were serological outliers (e.g., in groups 1B-1D, 3A and 4A). In the groups 2A-2D, 91% (48/53) of subjects with DU were obtained. In groups 3A and 3B, 57% (26/46) of those with AG and 18% (9/50) of those diagnosed with PA were obtained. In groups 4A-4C, 72% (36/50) of those with PA and 22% (10/46) of those with AG were obtained. This overlap between the AG and PA group is not surprising since they represent the same disease, but on a different point of the time scale. Furthermore, an individual overlap was indicated by the 14% of the subjects with AG found in group 2. Otherwise the overlaps appeared small. The overall sensitivity to detect gastritis was 98% (146/149) (95% CI 94-100%) and the specificity 84% (42/50) (95% CI 71-93%).

Table 1						
Serological Group	0.041667	1B	1C	1D	0.083333333	2B
Selection Pathway						
HK	<10 (2.0)	<10 (2.6)	<10 (1.0)	>10 (24.0)	<10 (4.2)	<10 (7.1)
HP	<5 (1.0)	<5 (2.0)	<5 (1.0)	<5 (2.0)	>5 (46.0)	>5 (10.0)
PGI	30-150 (70)	<30 (17)	>150 (163)	>30 (43)	-136	-122
HP*PGI	-83	-20	-163	-129	>2000 (6755)	<2000 (1216)
PGI	-	-	-	-	-	>90
HP*PGI	-	-	-	-	-	-
Clinical Groups						
n=199	45	7	1	4	30	2
Normal (50)	42	3	1	2	0	0
DU (53)	2	0	0	0	23	2
AG (46)	0	1	0	1	7	0
PA (50)	1	3	0	1	0	0
Sex						
M 123 (62%)	35 (77.8%)	4 (57.1%)	1	2 (50%)	21 (70%)	1 (50%)
F 76 (38%)	10 (22.2%)	3 (42.9%)	-	2 (50%)	9 (30%)	1 (50%)
Age						
median 67	66	60	71	69	59	43
range 20-83	37-80	38-75	-	61-78	20-79	38/48

Table 1 (cont.)							
Serological Group	2C	2D	0.125	3B	0.166667	4B	4C
Selection Pathway							
HK	>10 (25.0)	>10 (10.3)	<10 (2.0)	>10 (42.0)	<10 (2.3)	>10 (67.0)	>10 (39.0)
HP	>5 (53.5)	>5 (11.4)	>5 (18.5)	>5 (17.0)	>5 (7.0)	>5 (6.0)	<5 (2.0)
PGI	-150	-148	-36	-23	-9	-4	<30 (8)
HP*PGI	>2000 (9232)	<2000 (1602)	<2000 (673)	<2000 (425)	<2000 (79)	<2000 (28)	<150 (8)
PGI	-	>90	<90	<90	<90	<90	-
HP*PGI	-	-	>150	>150	<150	<150	-
Clinical Groups							
n=199	22	2	24	15	5	11	31
Normal (50)	0	0	1	0	1	0	0
DU (53)	21	2	1	2	0	0	0
AG (46)	1	0	17	9	2	3	5
PA (50)	0	0	5	4	2	8	26
Sex							
M 123 (62%)	18 (81.8%)	1 (50%)	12 (50%)	6 (40%)	3 (60%)	5 (45.5%)	14 (45.2%)
F 76 (38%)	4 (18.2%)	1 (50%)	12 (50%)	9 (60%)	2 (40%)	6 (54.5%)	17 (54.8%)
Age							
median 67	53	58	70	68	72	73	70
range 20-83	23-79	48-68	40-83	34-82	54-82	54-82	44-81

FIGS. 3A-3D present the distribution profiles of the four major histomorphologically diagnosed groups identified by serology: group 1A, comprising 94% N, 4% DU and 2% PA; group 2 (A-D) comprising 86% DU and 14% AG; group 3 (A-B) comprising 66% AG, 23% PA, 8% DU, and 3% N; and group 4(A-C) comprising 77% PA, 21% AG, and 2% N. Together groups 3 and 4 comprised 95% of subjects in groups AG and PA (42% and 53%, respectively), 2% N, and 3% DU. A smaller group

represented by groups 1B-D in Table 1, comprised twelve subjects of whom 50% belonged to group N, but serologically they were outliers. The remaining six subjects in these subgroups had either AG (17%) or PA (33%).

EXAMPLE 8

Serologic diagnosis in a sample of the general population

A sample of 483 subjects (age 37 to 85 years) randomly selected from a general population in Sweden was previously examined with EGD with biopsy and blood sampling. Fifty percent (243/483) had a normal gastric mucosa and the remaining had gastritis (Borch, K., et al., Dig Dis Sci, 2000, 45: 1322-29). In the present study sera from this population were analysed and grouped according to the scheme in FIG. 2 and the outcome compared with that of the histomorphological diagnosis. Table 2 shows the serologic grouping of the general population sample. The scheme for serologic diagnosis given in FIG. 2 was applied to the serologic data. The grouping resulted in the serologic subgroups 1A - 4C. The discriminating levels are indicated for the selection pathway in each subgroup and the median value of each group is indicated in parenthesis. The distributions of the histomorphologically diagnosed groups, sex, and age in each serologic subgroup are presented. The morphological diagnose were normal (0), non-atrophic antrum predominant gastritis (1), atrophic antrum predominant gastritis (2), non-atrophic pangastritis (3), atrophic pangastritis (4), non-atrophic corpus predominant gastritis (5), and atrophic corpus predominant gastritis (6).

Table 2						
Serological Group	0.041666667	1B	1C	1D	0.08333333	2B
Selection Pathway						
HK	<10 (1.8)	<10 (1.5)	<10 (1.6)	>10 (17.8)	<10 (2.6)	<10 (2.3)
HP	<5 (0.9)	<5 (1.1)	<5 (1.2)	<5 (1.1)	>5 (30.3)	>5 (11.2)
PGI	30-150 (63)	<30 (22)	>150 (227)	>30 (64)	-96	-107
HP*PGI	-56	-22	-248	-60	>2000 (2691)	<2000 (1189)
PGI	-	-	-	-	-	>90
HP*PGI	-	-	-	-	-	-
Histomorphological Diagnosis						
n=483	225	18	7	25	38	22
0 (normal) 243 (50.3%)	196	16	5	20	-	2
1 (ant) 20 (4.1%)	5	-	1	1	3	1
2 (ant a) 87 (18.1%)	12	-	1	-	14	5
3 (pan) 74 (15.3%)	5	-	-	1	14	13
4 (pan a) 14 (2.9%)	0	-	-	-	6	-
5 (corp) 13 (2.7%)	7	1	-	2	-	1
6 (corp a) 32 (6.6%)	0	1	-	1	1	-
Sex						
M 261 (54%)	127 (56.4%)	7 (38.9%)	6 (85.7%)	11 (44.0%)	23 (60.5%)	16 (72.2%)
F 222 (46%)	98 (43.6%)	11 (61.1%)	1 (14.3%)	14 (56.0%)	15 (39.5%)	6 (27.3%)
Age						
median (65)	56	52	66	62	65	63
range (37-85)	37-81	38-74	47-85	37-78	44-81	41-74

Table 2 (cont.)

Serologic Group	2C	2D	0.125	3B	0.1666667	4B	4C
Selection Pathway							
HK	>10 (30.3)	>10 (69.4)	<10 (2.0)	>10 (31.1)	<10 (2.2)	>10 (70.5)	>10 (68.5)
HP	>5 (28.4)	>5 (12.2)	>5 (16.4)	>5 (18.8)	>5 (7.4)	>5 (7.3)	<5 (1.5)
PGI	-141	-112	-61	-49	-8	-9	<30 (8)
HP*PGI	>2000 (4140)	<2000 (1318)	<2000 (953)	<2000 (805)	<2000 (62)	<2000 (61)	<150 (18)
PGI	-	>90	<90	<90	<90	<90	-
HP*PGI	-	-	>150	>150	<150	<150	-
Histomorphological Diagnosis							
n=483	10	3	95	28	2	4	6
0 (normal) 243 (50.3%)	-	-	4	-	-	-	-
1(ant) 20 (4.1%)	1	-	8	-	-	-	-
2 (ant a) 87 (18.1%)	3	2	39	11	-	-	-
3 (pan) 74 (15.3%)	5	1	26	9	-	-	-
4 (pan a) 14 (2.9%)	1	-	5	2	-	-	-
5 (corp) 13 (2.7%)	-	-	1	-	-	-	1
6 (corp a) 32 (6.6%)	-	-	12	6	2	4	5
Sex							
M 261 (54%)	7 (70%)	3 (100%)	44 (46.3%)	12 (43%)	2 (100%)	2 (50%)	1 (17%)
F 222 (46%)	3 (30%)	-	51 (53.7%)	16 (57%)	-	2 (50%)	5 (83%)
Age							
median (65)	63	67	63	68	75	76	72
range (37-85)	44-73	58-72	38-80	43-80	47-82	69-80	50-80

In group 1A, 87% (196/225) of the subjects exhibited serologic values indicating a normal gastric mucosa. Seven percent (16/243) of the subjects with normal gastric mucosa had low levels of PGI and were obtained in group 1B, while 8% (20/243) were obtained in group 1D due to increased levels of H,K-ATPase antibodies. Six percent (29/483) of the population sample diagnosed as having gastritis of any type were obtained in group 1A.

In groups 2A-2D, 15% (73/483) were obtained by means of the serologic grouping procedure (Table 2). In groups 2A-2D only two subjects had normal mucosa. The dominating morphological diagnoses in groups 2A-2D were antrum predominant gastritis with atrophy (24 subjects) and pangastritis without atrophy (33 subjects). Subjects in groups 3A-3B exhibited a positive serology for H. pylori. In group 3A, 20% (95/483) of the population were obtained and the dominating morphological diagnoses were antrum predominant gastritis with atrophy (39 subjects), pangastritis without atrophy (26 subjects), and pangastritis with atrophy or corpus predominant atrophic gastritis (17 subjects). In groups 4A-4C, the PGI values were generally low which indicated a more pronounced corpus atrophy. Only twelve subjects were found in these groups and they had corpus predominant gastritis with atrophy. The overall sensitivity to detect gastritis serologically in the population was 88% (211/240) (95% CI 83-92%) and the specificity 81% (196/243) (95% CI 75-85%).

The distribution profile according to serology in the four major groups of the sample of the general population is presented in FIGS. 4A-4D. The distribution profiles of the histomorphologically diagnosed groups in the serologic groups 1A, 2(A-D), 3(A-B) and 4(A-C) are presented. The morphological diagnoses were normal (0), non-atrophic antrum predominant gastritis (1), atrophic antrum predominant gastritis (2), non-atrophic pangastritis (3), atrophic pangastritis (4), non-atrophic corpus predominant gastritis (5), and atrophic corpus predominant gastritis (6).

In group 1A, the morphologically assessed normals comprised 87% (196/225). In group 2, antrum gastritis with atrophy, comprising 33% (24/73), and pangastritis, comprising 45% (33/73), were the dominating groups. In group 3 (A-B), antrum gastritis

with atrophy, 40% (50/123), pangastritis, 28% (35/123), and atrophic corpus gastritis, 15% (18/123) were the dominating groups. In group 4 (A-C), atrophic corpus gastritis comprised 92% (11/12) and non-atrophic corpus gastritis the remaining 8% (1/12).

The dominating morphological diagnoses in groups 3 plus 4 were atrophic antrum predominant gastritis, 35% (50/145), pangastritis, 24% (35/145), and corpus predominant atrophic gastritis, 20% (29/145). These three subgroups comprised 79% (114/145) of the subjects in groups 3 plus 4. The prevalence of atrophic corpus predominant gastritis was 7% (32/483) in the population, 91% (29/32) of these were obtained in groups 3 plus 4.

Although the preferred embodiment of the method and kit of the invention has been described above in some detail, it should be appreciated that a variety of embodiments will be readily apparent to one skilled in the art. The description of the method and kit of this invention is not intended to be limiting to this invention, but is merely illustrative of the preferred embodiment.

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We claim:

1. A method for evaluating a blood sample from a patient to assess the likelihood that the patient has gastritis, the method comprising assaying the blood sample for the presence of antibodies specific for H,K-ATPase, antibodies specific for Helicobacter pylori, and the concentration of pepsinogen I, wherein the presence of H,K-ATPase antibodies, Helicobacter pylori antibodies, and altered pepsinogen I concentration as compared with pepsinogen I concentration from an individual without gastritis, is indicative of the patient having gastritis.

2. A method for screening for gastritis in a mammal suspected of suffering from gastritis comprising determining the level of H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I in a biological sample from a mammal suspected of suffering from gastritis, and comparing the level of H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I to levels in normal mammals of the same species, wherein altered levels in the sample compared to the levels in normal mammals of the same species is indicative of gastritis.

3. A method for diagnosing gastritis in a mammal suspected of suffering from gastritis comprising determining the levels of at least two indicators in a biological sample from a mammalian patient, the indicators selected from the group consisting of H,K-ATPase antibodies, Helicobacter pylori antibodies, pepsinogen I, and the level of pepsinogen I multiplied by the level of Helicobacter pylori antibodies; and comparing the levels of the indicators in the patient sample to levels of the same indicators in normal mammals of the same species, wherein levels of at least two indicators in the patient sample which differ significantly from the level of the same indicators in normal mammals of the same species indicates the patient has gastritis.

4. A method for screening for gastritis in a mammal suspected of suffering from gastritis comprising determining the levels of at least two indicators in a biological sample from a mammalian patient, the indicators selected from the group consisting of H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I and comparing the levels of indicators in the patient sample to levels of the same indicators in normal mammals of the same species, wherein levels of at least two indicators in the patient sample which differ significantly from levels of the same indicators in normal mammals of the same species is indicative of gastritis.

5. The method of claim 4, wherein the levels of all three indicators are determined.

6. The method of claim 4, wherein the step of determining the levels of at least two indicators comprises performing immunoassays for detecting the indicators.

7. The method of claim 4, wherein the group of indicators further includes an additional indicator comprising the level of pepsinogen I multiplied by the level of Helicobacter pylori antibodies, and wherein the level of this additional indicator is compared to a standard.

8. The method of claim 4, wherein levels of H,K-ATPase antibodies, and Helicobacter pylori antibodies which are significantly higher than the levels in normal mammals of the same species is indicative of gastritis.

9. A kit for screening for gastritis comprising reagents suitable for detecting H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I.

10. The kit of claim 9, wherein the reagents comprise pepsinogen I antibodies, H,K-ATPase and Helicobacter pylori proteins or peptides thereof.

11. The kit of claim 9, wherein the reagents comprise pepsinogen I, H,K-ATPase, and Helicobacter pylori antigens immobilized on a solid support.

12. The kit of claim 11, further comprising labeled anti-human antibodies.

13. The kit of claim 9, wherein the reagents are provided in amounts sufficient to perform substantially equal numbers of assays to detect H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I concentration.

ABSTRACT

A screening method for gastritis is provided. The method involves the serological measurement of at least two of the following analytes: H,K-ATPase antibodies, Helicobacter pylori antibodies and the concentration of pepsinogen I; and the evaluation of the results in comparison with results from normal individuals. The evaluation scheme provides an initial, non-invasive identification of individuals with various forms of gastritis.

Fig. 1

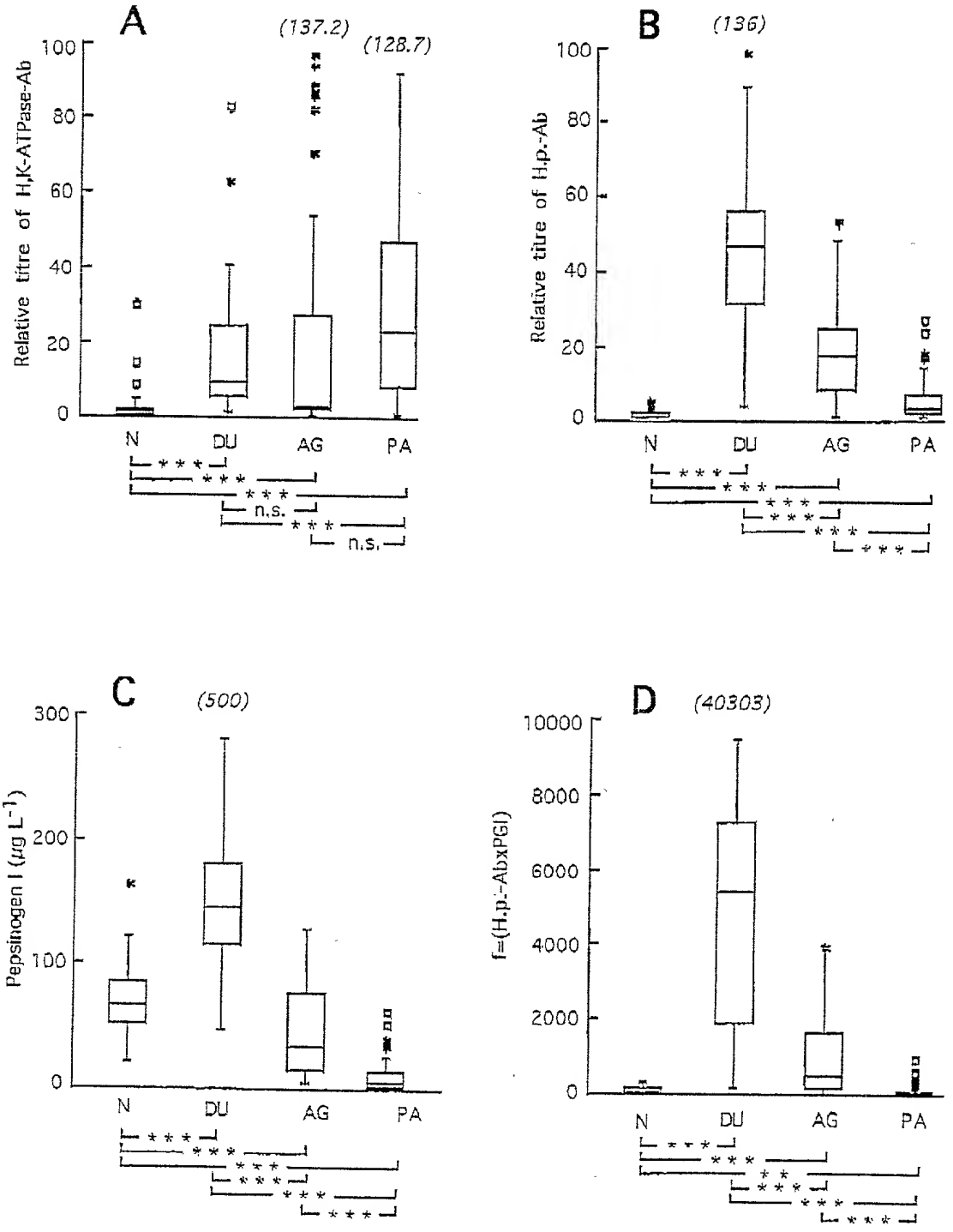
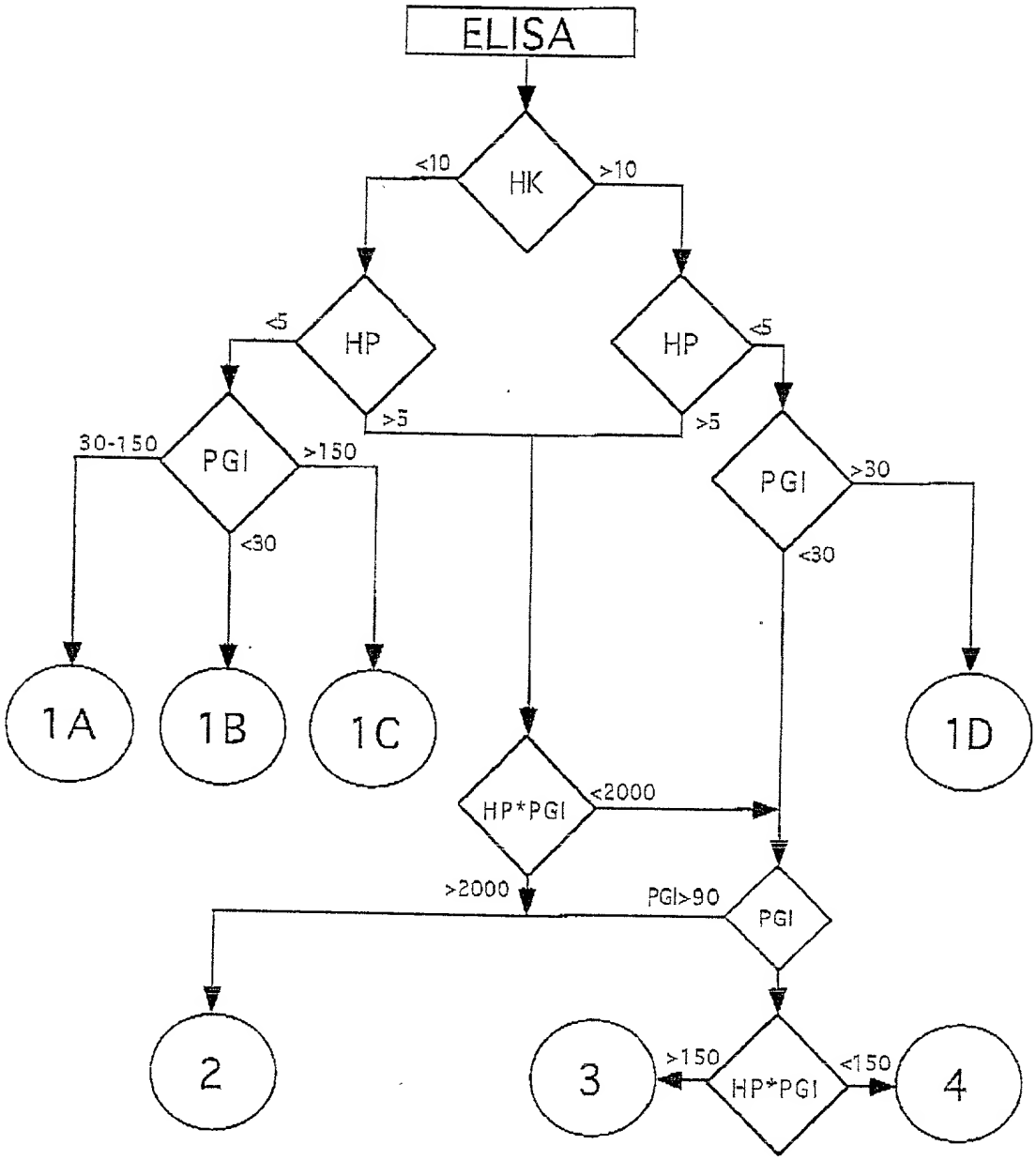

A
(137.2)
(128.7)
Relative titre of H,K-ATPase-Ab
100
80
60
40
20
0
N
DU
AG
PA
n.s.
n.s.
B
(136)
Relative titre of H.p.-Ab
C
(500)
Pepsinogen I (µg L-1)
300
200
100
0
D
(40303)
f=(H.p.-AbxPGI)
10000
8000
6000
4000
2000

Fig. 2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HP
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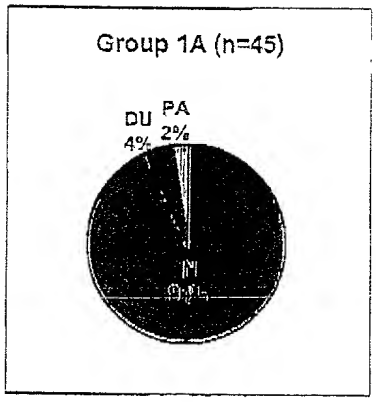
>30
<30
1A
1B
1C
1D
HP*PGI
<2000
>2000
PGI>90
PGI
2
3
>150
HP*PGI
<150
4

Fig.3

A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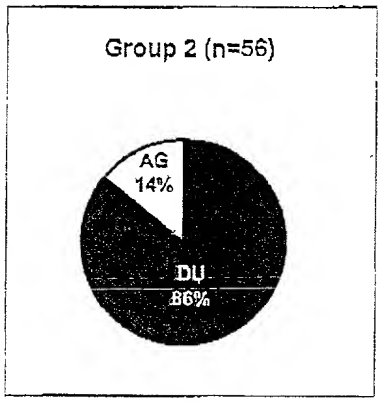
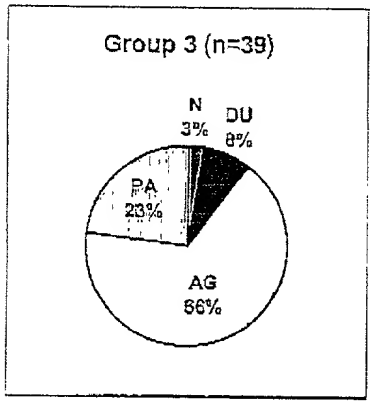
DU 4%
PA 2%
N

B


Group 2 (n=56)
AG 14%
DU 86%

C


Group 3 (n=39)
N 3%
DU 8%
PA 23%
AG 66%

D

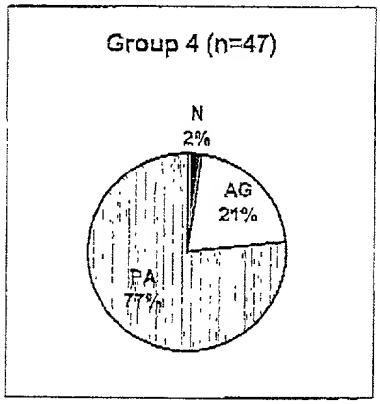
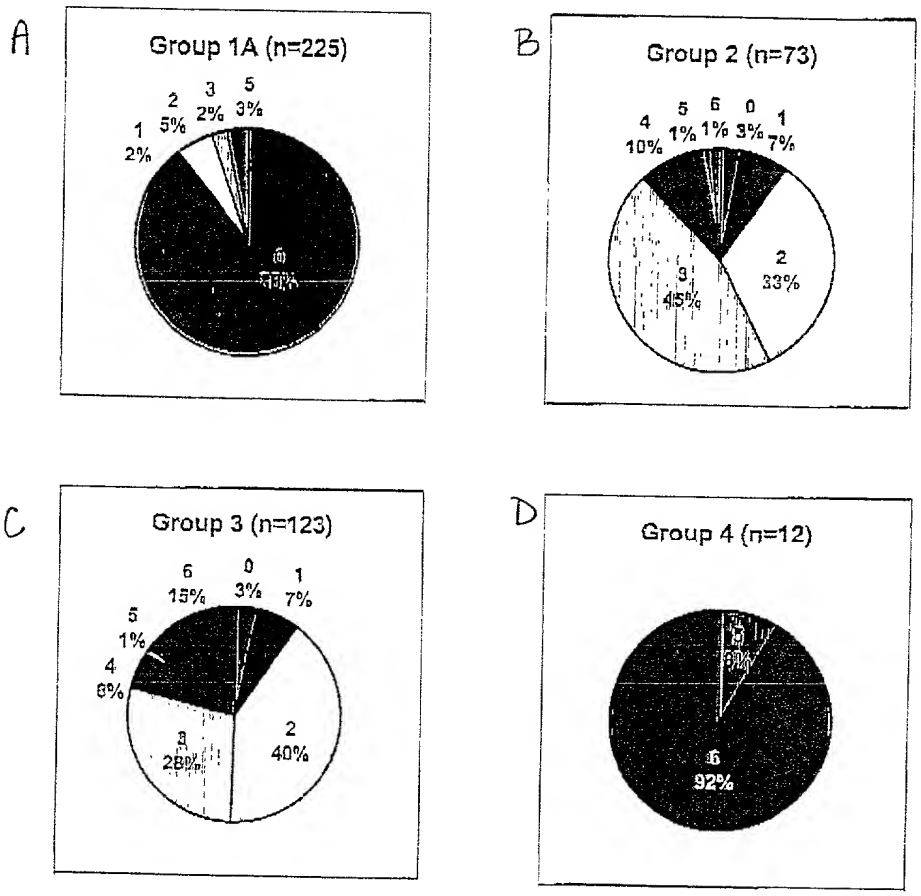

Group 4 (n=47)
N 2%
AG 21%
PA 77%

Fig.4


A
Group 1A (n=225)
5 3%
3 2%
2 5%
1 2%
6
B
Group 2 (n=73)
5 1%
6 1%
0 3%
1 7%
4 10%
2 33%
3 45%
C
Group 3 (n=123)
6 15%
0 3%
1 7%
5 1%
4 6%
2 40%
3 28%
D
Group 4 (n=12)
6 92%

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventors, we hereby declare that:

Our citizenship, residence and post office address are as listed below next to our name.

We believe we are the original, firsts and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled: Screening Method for Gastritis

the specification of which

(a) [X] is attached hereto.

(b) [ ] was filed on_____ as Application Serial No. _____ and was amended on _____.

(c) [ ] was described and claimed in International Application No. _____ filed on _____ and amended on _____.

Acknowledgment of Duty of Disclosure

I hereby state that I have reviewed and understood the content of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56(a).

35 U.S.C. § 120

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status)(patented,pending,abandoned)	(Patent No. if applicable)
(Application Serial No.)	(Filing Date)	(Status)(patented,pending,abandoned)	(Patent No. if applicable)

Power of Attorney

I hereby appoint Carl Oppedahl, PTO Reg. NO. 32,746, Marina T. Larson, PTO Reg. No. 32,038, and Nancy J. Parsons, PTO Reg. No. 40,364 of the firm of OPPEDAHL & LARSON LLP, having office at P.O. Box 5068, Dillon, CO 80435-5068 as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

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Claim for Priority

I hereby claim foreign priority benefits under 35 U.S.C. § 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign applications for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

EARLIEST FOREIGN APPLICATION(S), FILED WITHIN TWELVE MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION				
COUNTRY	APPLICATION NO.	DATE OF FILING (day/month/year)	DATE OF ISSUE (day/month/year)	PRIORITY CLAIMED / CERTIFIED COPY ATTACHED
				YES[ ] NO[ ] YES[ ] NO[ ]
FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION				
COUNTRY	APPLICATION NO.	DATE OF FILING (day/month/year)	DATE OF ISSUE (day/month/year)	

Provisional Application

I hereby claim the benefit under 35 U.S.C § 119(e) of any United States provisional application(s) listed below.

_____ (application number) _____ (filing date)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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DATE Linköping 2000-10-03		SIGNATURE	

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[ ] Signature by Administrator(trix) or legal representative for deceased or incapacitated inventor. Number of Pages ___.
[ ] Signature for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR § 1.47. Number of Pages ___.

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POST OFFICE ADDRESS		CITY	STATE/COUNTRY ZIP CODE
DATE		SIGNATURE	
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POST OFFICE ADDRESS		CITY	STATE/COUNTRY ZIP CODE
DATE		SIGNATURE	
NAME OF FIFTH INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY OF RESIDENCE	STATE OR COUNTRY OF RESIDENCE	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS		CITY	STATE/COUNTRY ZIP CODE
DATE		SIGNATURE	